PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Elucidate Traffic Patterns Of Ihn Virus In The Columbia River Basin

BPA project number: 20056
Contract renewal date (mm/yyyy): ☐ **Multiple actions?**

Business name of agency, institution or organization requesting funding
USGS-BRD, Western Fisheries Research Center

Business acronym (if appropriate) USGS-WFRC

Proposal contact person or principal investigator:

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NPPC Program Measure Number(s) which this project addresses
2.1, 4.1, 7.2A.6, 7.2D.4, 7.2D.6, 7.2D.7

FWS/NMFS Biological Opinion Number(s) which this project addresses

Other planning document references
1994 Final Report of the research priority sub-committee of the PNFHPC

Short description
RNase protection technology will be used to survey the genetic types of IHN virus throughout the Columbia Basin, to identify sources of disease outbreaks, and to infer viral traffic patterns in an effort to reduce the impact of IHNV on basin salmonids.

Target species
Oncorhynchus mykiss, O. tshawytscha, O. nerka

Section 2. Sorting and evaluation

Subbasin
Systemwide

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more	If your project fits either of these	Mark one or more categories

caucus	processes, mark one or both	
☒ Anadromous fish ☒ Resident fish ☐ Wildlife	☐ Multi-year (milestone-based evaluation) ☐ Watershed project evaluation	☐ Watershed councils/model watersheds ☐ Information dissemination ☐ Operation & maintenance ☐ New construction ☒ Research & monitoring ☐ Implementation & management ☐ Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Analyze 81 IHN virus isolates by RNase protection assays to obtain genetic fingerprints of IHNV types throughout the Columbia River basin	a	Assay 20 IHNV isolates from the lower Columbia sub-region
		b	Assay 14 IHNV isolates from the lower mid-Columbia sub-region
		c	Assay 19 IHNV isolates from the upper mid-Columbia sub-region
		d	Assay 22 isolates from the lower Snake sub-region

		e	Assay 6 isolates from the upper Snake sub-region
2	Obtain additional IHNV isolates to get a complete representation of IHNV throughout the basin.	a	Obtain additional isolates as possible, through collaborations with colleagues
		b	Assay additional isolates as in objective 1
3	Begin analysis of all data using "Molecular Analyst: Fingerprint Analysis" computer software	a	Enter all genetic fingerprint patterns into the program to create a database of IHNV genetic types throughout the Columbia River Basin
		b	Search the database for related patterns to infer virus traffic and sources of disease outbreaks in the basin

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	10/1999	10/2000	Number of IHNV isolates assayed		60
2	10/1999	10/2000	Number of additional IHNV isolates obtained and assayed		10
3	10/1999	10/2000	Number of fingerprint patterns archived and traffic links identified		30
				Total	100

Schedule constraints

Completion date
2000

Section 5. Budget

FY99 project budget (BPA obligated):

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	One full-time GS9 technician	43	32,478
Fringe benefits	WFRC rate 25%	11	8,120
Supplies, materials, non-expendable property	radioisotope and supplies	13	10,000
Operations & maintenance	fedex charges for collaborators	0.5	400
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	one computer dedicated to the database	2.5	2000
NEPA costs			
Construction-related support			

PIT tags	# of tags:		
Travel	one meeting and 1-2 field trips	2	1500
Indirect costs	WFRC rate 38%	27.5	20,709
Subcontractor			
Other			
	TOTAL BPA FY2000 BUDGET REQUEST		75,207

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
USGS-WFRC	0.3 FTE of PI	14	17,300
USGS-WFRC	0.3 FTE of a GS11 technician	10	12,180
USGS-WFRC	annual radiation license	4.5	5,500
USGS-WFRC	molecular analyst software programs and scanner	10	12,000
	Total project cost (including BPA portion)		122,187

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget				

Section 6. References

Watershed?	Reference
	Anderson ED, HM Engelking, EJ Emmenegger, and G Kurath. 1996. Genetic diversity of field isolates of infectious hematopoietic necrosis virus: Analysis by ribonuclease protection assay. Abstract, Western Fish Disease Workshop, Corvallis, Oregon.
	Anderson ED, HM Engelking, EJ Emmenegger, and G Kurath. Molecular epizootiology of an IHN virus outbreak in wild kokanee demonstrated transmission to nearby heatchery stocks. (In preparation)
	Anderson ED, G Traxler, and G Kurath. Genetic diversity of British Columbian IHN virus isolates include the IHN strain associated with epizootics in farmed Atlantic salmon. (In preparation)
	Busch RA. 1983. Viral disease considerations in the commercial trout industry in Idaho. pp.84-100 *In* JC Leong and TY Barila, eds. Workshop on viral diseases of salmonid fishes in the Columbia River Basin. Bonneville Power Administration, Special publication, Portland, Oregon.
	Emmenegger EJ, ED Anderson, G Kurath. 1996. Rapid genetic analysis of IHNV strains by Rnase protection assay. Abstract, Western Fish Disease Workshop, Corvallis, Oregon.
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	Hsu YL, HM Engelking, and JC Leong. 1986. Occurrence of different types of infectious hematopoietic necrosis virus in fish. App. Env. Micro. 52:1353-1361.
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	development of RNase protection assays for rapid assessment of genetic variation. Vet. Res. 26:477-485.
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	Morse SS. 1994. The viruses of the future? Emerging viruses and evolution. pp.325-335 *In* The evolutionary biology of viruses. SS Morse Ed., Raven Press, New York
	Nichol ST, JE Rowe, and JR Winton. 1995. Molecular epizootiology and evolution of the glycoprotein and non-virion genes of infectious hematopoietic necrosis virus, a fish rhabdovirus. Virus Res. 38:159-173.
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	Ristow SS and J Arnzen de Avila. 1991. Monoclonal antibodies to the glycoprotein and nucleoprotein of infectious hematopoietic necrosis virus (IHNV) reveal differences among isolates of the virus by fluorescence, neutralization, and electrophoresis. Dis. Aquat. Org. 11:105-115.
	Rucker RR, WJ Whipple, JR Parvin, and CA Evans. 1953. A contagious disease of salmon possibly of virus origin. U.S. Fish Wildlife Serv. Fish. Bull. 54:35-46.
	Sleat DE and P Palukaitis. 1990. Site-directed mutagenesis of a plant viral satellite RNA changes its phenotype from ameliorative to necrogenic. Proc. Natl. Acad. Sci. 87:2946-2950.
	Stewart BC, R Brunson, SE La Patra, P Reno, T Sheldrake, and JR Winton. 1994. Final report of the research priority subcommittee of the Pacific Northwest Fish Health Protection Committee.
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PART II - NARRATIVE

Section 7. Abstract

Ribonuclease protection analysis technology has been developed for differentiating field isolates of IHN virus by genetic fingerprinting. In two localized studies of IHNV field isolates from the Deschutes and upper Snake Rivers these fingerprints provided data that documented transmission of IHNV from wild fish to hatchery populations, and identified local and distant viral sources of disease outbreaks. The high resolution provided by this new technology indicated more complex and dynamic virus traffic patterns than were previously understood.

This one year innovative research proposal involves a systemwide survey of genetic types of IHNV throughout the Columbia River Basin, using a collection of 81 IHNV isolates already compiled by collaboration with Columbia Basin fish health professionals. This collection represents 27 locations in 15 different sub-basins over 25 years, including virus isolated from wild and hatchery fish of different species and life stages, and ESA listed chinook. Fingerprint analysis by a computerized database can identify links indicating sources of disease outbreaks, frequency of virus introductions, and directions of virus traffic. This information will assist hatchery managers design strategies to eliminate sources of IHNV, and help resource managers avoid risks associated with movements of fish stocks and their pathogens. The ultimate goal of the research is to improve overall health of wild and hatchery salmonids by reducing the impact of IHNV disease in the Columbia Basin. Relative to the 1994 FWP, this proposal addresses the systemwide goal of creating a healthy Columbia Basin, and specific fish health measures 7.2A.6, 7.2D.4, 7.2D.6, and 7.2D.7.

Section 8. Project description

a. Technical and/or scientific background

The term " viral traffic" refers to the "transfer and dissemination of viruses to new host populations" (Morse, 1991; Morse 1994). Within the Columbia River Basin it is known that numerous salmonid fish populations harbor infectious hematopoietic necrosis (IHN) virus, and our preliminary studies have indicated that there is far more virus traffic between these populations than was previously recognized. We have shown that at a single location there can be frequent displacement of the dominant virus strain by introduction of strains from outside sources, and in certain cases we have been able to identify the sources of new virus strains causing disease outbreaks. We have also documented transmission of IHN virus from a wild host population to hatchery populations. It is logical to assume that these and other disease phenomena are occurring at various levels throughout the basin, and that they contribute significantly to the losses of salmon and trout to IHNV. Therefore, we propose to expand our current localized information to assess IHNV traffic throughout the Columbia River Basin, using both historical and current collections of IHNV isolates. This entails a survey of the genetic types of IHNV that exist throughout the basin, to create a database of baseline information against which newly introduced virus strains or strains causing novel disease outbreaks can be compared. Analyses of this database would provide a large scale picture of the sources and sinks of virus movement throughout the basin, and the traffic patterns thus elucidated would be correlated with the incidences of disease outbreaks. This epizootiological understanding could then be used at fish culture facilities and in fish stock management decisions to reduce the impact of IHNV on both hatchery and wild fish populations.

Over the last 25 years IHN virus has been responsible for numerous disease outbreaks in both cultured and wild juvenile fish (Wolf, 1988). Since the first description of IHN virus in 1953 (Rucker et al.), many IHNV isolates have been collected from both hatchery and wild fish hosts. Studies of these isolates have shown that there are many distinct strains of IHN virus that differ in their virulence, biological characteristics, host specificities, and serological reactions. Due to the impact of IHNV epizootics in salmon and trout culture facilities, this virus has been well characterized at both the biological and molecular levels.

IHNV is a member of the Rhabdovirus family, and is the type member of the newly established genus,"*Novirhabdovirus*", which includes many of the rhabdoviruses that infect fish hosts (Walker et.al.,

in press). As a rhabdovirus IHNV has structural and genetic similarities with rabies virus. The genetic component of IHNV is a molecule of single-stranded RNA that is approximately 11,000 nucleotides long and contains six genes. These genes have all been characterized, and together they encode all the functions necessary for virus replication and transmission. At the fundamental level, nucleotide sequence differences between the RNA genes of different virus strains are responsible for the biological differences between these strains. It is known that the genetic basis responsible for differences in biological traits such as virulence can be as subtle as single nucleotide differences in relevant viral genes (Kawaoka and Webster, 1988; Sleat and Palukaitis, 1990). From a practical standpoint, the diversity of IHNV strains in nature can be used to produce genetic fingerprints that track how the virus moves and changes both spatially and temporally.

Several studies have documented IHNV genetic diversity using various methods to distinguish between virus strains. The first survey of IHNV diversity was carried out by Hsu et al. (1986) using differences in the electrophoretic mobility patterns of the viral proteins to group 71 IHNV isolates into five types. Subsequently a monoclonal antibody reagent, 105B, was applied to the same 71 IHNV isolates and shown to react with near perfect specificity with strains designated type 2 by the previous method (Ristow and Arnzen, 1989). Both of these valuable tools are still in use today, but they have relatively low resolution in that they differentiate all IHNV strains into a small number of possible types. Later studies used panels of several monoclonal or polyclonal antisera reagents to group IHNV isolates by patterns of reactivity (La Patra et.al., 1994; Ristow and Arnzen de Avila, 1991; Winton et al., 1988). Again, although this provided valuable information, the resolution is not high. In contrast, the RNase T1 fingerprinting method used by Oshima et al. (1995) to characterize 26 IHNV isolates is higher resolution, but the labor-intensity of this method precludes its use on a large scale. The most recent study of IHNV diversity used nucleotide sequence analyses of 12 IHNV strains to determine the relationships between the strains by phylogenetic analyses (Nichol et al., 1995). Although sequence data is the highest resolution possible for distinguishing strains, again it is labor-intensive in that they determined approximately 2000 bases of sequence for each strain. Each of the IHNV diversity studies described above arrived at the same conclusion: that IHNV strain relatedness tends to correlate with geographic origin, and not with host species or date of isolation. Thus, these methods found that virus isolates generally grouped according to watersheds, and suggested that the IHN virus populations have evolved along with the populations of their salmonid hosts.

For our studies of IHNV genetic diversity we have developed an RNase protection assay (RPA) system to distinguish IHNV strains on a genetic basis that is high resolution, and can assess multiple strains simultaneously (Kurath et al., 1995). The RPA system assays each IHNV isolate with probes that detect sequence differences in three of the six viral genes (see section f). The result is fingerprint banding patterns that are diagnostic for a certain genetic type of IHNV. Within sets of multiple IHNV isolates, comparison of the fingerprint patterns identifies the following possible relationships; a) isolates with identical patterns are genetically extremely similar, b) isolates with similar but non-identical banding patterns are related, and c) isolates with patterns that share no bands are most distantly related. When this system was first developed we tested it on 12 IHNV strains that had been previously characterized (Nichol et al., 1995), and found that the relationships determined by RPA correlated extremely well with the known relationships determined by gene sequence determination and phylogenetic analyses (Kurath, Anderson, and Emmenegger, unpublished data).

The first application of RPA technology to IHNV field isolates was initiated in 1996 through a collaboration with Dr. H. M. Engelking of the Oregon Department of Fish and Wildlife, Corvallis, Oregon. This first study involved 20 IHNV field isolates from the Deschutes River subbasin. The impetus for the study was dramatic die-offs of wild yearling kokanee in Lake Billy Chinook that began in 1991 and continued through 1995. Pathology investigations by Dr. Engelking indicated that these die-offs were associated with an outbreak of IHNV, but this resident kokanee population had been known to carry IHNV since the late 1970's. Therefore, the questions were: (a) whether these epizootics were due to the introduction of a new and more virulent virus strain, and (b) if so, did that new strain come from Round Butte Hatchery, which lies directly below Lake Billy Chinook and has a long history of IHNV disease incidence. Comparisons of the RPA fingerprint patterns of IHNV isolates from the kokanee die-offs with earlier isolates from the same kokanee population indicated that indeed a new strain of IHNV had been introduced. The new strain was first detected in Metolius River spawning kokanee, upstream of Lake Billy Chinook, in 1988. RPA analyses of several IHNV isolates from Round Butte Hatchery then yielded surprising information in that there were seven different virus types between 1981 and 1995. This

indicated that the dominant virus strains at the hatchery had been displaced by a new strain at least six times during the last 14 years. The virus type matching the fingerprint of the kokanee die-offs in Lake Billy Chinook was first evident at Round Butte Hatchery in 1991, and it persisted through 1995. Due to the detection of this new strain first in the kokanee in 1988 and its absence at Round Butte Hatchery until 1991, we can conclude that the new strain did not come from the hatchery, but that it was transmitted from the wild kokanee population to the hatchery during the die-offs (Anderson et. al., in preparation).

This first study had several important outcomes. First, it demonstrated that the level of resolution of our new RPA technology was appropriate for analyzing the level of genetic diversity that exists in field isolates of IHNV. RPA could thus distinguish field isolates into many different genetic fingerprint types. Second, it is the first clearly documented case of transmission of a virulent virus strain from a wild fish population to hatchery fish. Finally, the finding of frequent displacement of virus stains at a single site provides many insights. Using the previous protein pattern typing system the IHNV at Round Butte had been characterized as type 1, with very infrequent detection of type 2 strains. Therefore it was logically assumed that the high frequency of IHNV detected at the hatchery was due to the persistent presence of a single type 1 strain of IHNV. With the higher resolution afforded by RPA technology, we have shown that the presence of IHNV at Round Butte Hatchery is a much more complex and dynamic phenomenon than was previously recognized. Since it involves frequent introduction of new virus strains, identification of the sources of these strains could lead to modifications in management practices to reduce or eliminate these introductions. One of the sources was identified in this study as wild fish upstream in the hatchery water supply. In subsequent monitoring of the virus types at Round Butte Hatchery since 1995 we have observed another major shift in which the kokanee strain was displaced by a new strain that matched the RPA fingerprint patterns of isolates from Bonneville and Little White Salmon hatcheries on the lower mid-Columbia mainstem below the Deschutes River (Kurath and Engelking, unpublished data). In a recent analysis we have found a match of the virus type that was dominant at Round Butte Hatchery in 1989 and 1990 with a 1986 virus isolate from Lookingglass Hatchery on the Grande Ronde sub-basin, upstream on the lower Snake River from the Deschutes River [G. Kurath and W. Groberg (ODFW), unpublished data]. It is links such as these that can be used to determine the directions of virus traffic. We anticipate that a thorough analysis of IHNV types throughout the Columbia River Basin will generate many more unexpected links, and hopefully lead us to hypotheses about general mechanisms of virus movements in the basin that can be used for management decision purposes.

Preliminary results of the RPA method and this first field study were presented at the Western Fish Disease Workshop in Corvallis in 1996 (Emmenegger et al., 1996; Anderson et. al., 1996). The response from many fish health professionals at the workshop indicated a great deal of interest in our new technology, and many collaborations have been established in which these colleagues provide biologically relevant sets of IHNV isolates and we assay them by RPA to answer different questions about IHNV at the genetic level. We have recently completed a study done in collaboration with Dr. G. Traxler of the Canadian Department of Fisheries and Oceans, in which we analyzed 48 IHNV isolates from throughout British Columbia and compared them with virus isolates from recent IHNV outbreaks in Atlantic salmon netpens (Anderson et al., in preparation). In this study the 48 isolates were resolved into 24 fingerprint groups, and it was shown that the virus type associated with the netpen epizootics is genetically similar to local strains, indicating that it was not newly introduced with the Atlantic salmon. In another completed study done in collaboration with Dr. T. Meyers of the Alaska Department of Fish and Game, 42 IHNV isolates representing the geographic diversity of IHNV in Alaska over a 19 year time span were resolved into 36 fingerprint types, and the links identified showed general geographic and temporal proximity (Emmenegger et al., in preparation).

In a third collaborative study, with Dr. S. La Patra of Clear Springs Inc., we have focused on IHNV isolates from four trout farm facilities within a twelve mile stretch of the upper Snake River sub-basin. RPA analyses of 43 isolates from these facilites from 1990-1992 confirmed and extended the earlier report of high genetic diversity (La Patra et. al., 1994) in that 27 different genetic fingerprint types were identified. Additional analyses of 32 isolates from 1997-1998 show that this diversity has been maintained, so that at these sites numerous virus genetic types can co-circulate in a relatively stable manner (Troyer et al., in preparation). We have not compared these patterns to other isolates from elsewhere in the sub-basin.

This Snake River study and the initial study of IHNV in the Deschutes River comprise the two thorough studies of localized regions that we have carried out within the Columbia River Basin. From elsewhere in the basin we have collected numerous IHNV isolates from various fish health colleagues, but

only a very few of these have been analyzed. The systematic analysis of IHNV isolates representing the rest of the Columbia River Basin comprises the main objectives of this proposal.

b. Rationale and significance to Regional Programs

Within the Columbia River Basin the diseases that cause significant reductions in salmonid populations have been well characterized in the last two decades due to their prevalence and economic impact. Numerous populations of both wild and cultured salmonids in the basin are routinely surveyed and found to carry IHNV at varying levels of incidence. The 1994 final report of the research priority subcommittee of the Pacific Northwest Fish Health Protection Committee (Stewart et .al., 1994) states that in a survey of Pacific Northwest fish health workers IHNV was ranked as one of the four most significant diseases that impact production levels, and epizootiology was ranked as the second most important approach for preventing or controlling disease. Annual losses due to IHN virus have been estimated to range from 20-40% in the commercial trout industry (Busch 1983; La Patra et al., 1991) to as high as 25-90% in individual hatcheries (Winton 1991). At Lookingglass Hatchery in 1995 IHNV epizootics occurred in several raceways of ESA listed Imnaha chinook, and one raceway of fish was destroyed after the virus-induced mortality reached 30%. IHNV was detected in the same endangered stock in 1998 (W. Groberg, ODFW, personal communication), indicating that IHN is a continuing threat to these valuable fish. The ultimate goal of this proposal, to improve overall health of wild and hatchery salmonids by reducing the impact of IHNV disease in the Columbia Basin, will mitigate losses in place, and directly addresses the fish health measures in section 7.2 and the overall goal of the 1994 FWP, which is "a healthy Columbia Basin". The systemwide approach of this proposal also complements the FWP overall goal of "treating Columbia as a system". The database created in this proposal will provide valuable information to resource and hatchery managers now, and establishes a permanent baseline of data that will facilitate IHNV epizootiology throughout the basin long into the future.

c. Relationships to other projects

To our knowledge, there are no other research groups focusing on large scale genetic analyses of IHNV field isolates, either in the Columbia River basin or elsewhere throughout the range of the virus. The RNase protection assay (RPA) technology for rapid analyses of IHNV isolates on a genetic basis is currently only in use in our laboratory at the Western Fisheries Research Center. This is due to both the recent development of the technology, and to the need to use radioactive isotopes in these assays, which requires a current radiation license from the Nuclear Regulatory Commission. Although this license requirement will realistically preclude the transfer of this technology to most field laboratories, we have transferred the technology to many of our collaborators by having them come to our laboratory for three day RPA training sessions to learn both the theory and the hands-on experimental procedures involved with generating RPA fingerprints from their field isolates. To date, the following colleagues have come to the lab for RPA training sessions: Dr. H. M. Engelking and J. Kaufman, Oregon Department of Fish and Wildlife, Corvallis, Oregon; Dr. W. Groberg and S. Onjukaa, Oregon Department of Fish and Wildlife, La Grande, Oregon; Dr. G. Traxler, Dr. M. Kent, and S. St.-Hilaire, Canadian Department of Fisheries and Oceans; Dr. S. La Patra, Clear Springs Inc. In addition to taking time to come for these training visits, we have established ongoing collaborations with each of these these colleagues based on our mutual interest in understanding and controlling IHNV within the Columbia River basin. The products of these collaborations include the completed studies described in section (a) that are being prepared for publication, and ongoing interactions that advance the state of our knowledge of IHNV.

In addition to the collaborators listed above, the following colleagues have expressed genuine interest in the RPA technology by sending us valuable collections of IHNV isolates for analyses: R. Brunson, USFWS, Olympia, Wa.; K. True, USFWS, Anderson, Ca.; S. Gutenberger, USFWS, Underwood, Wa.; S. Lutz, Northwest Indian Fisheries Commission, Olympia, Wa; D. Ramsey, Rangen Inc., Id.; R. Hedrick, University of California, Davis, D. Keiser, Canadian Department of Fisheries and Oceans, B.C. As part of their official responsibilities most of these colleagues carry out surveys of spawning adults and epizootic juvenile fish for IHNV incidence. Thus our collaborations are exceptionally mutually beneficial in that their field isolate collection efforts can be utilized to gain more information through our interactions. This eliminates the cost of field isolate collection for us, makes optimal use of the professional field knowledge of these colleagues, and provides them with much more information for

understanding the virus diversity and traffic within their jurisdictions. Thus, although to our knowledge there are no related projects currently being funded under the FWP, extensive networks of interest and interaction are well established.

d. Project history (for ongoing projects)

e. Proposal objectives

This proposal is comprised of three specific work objectives listed below, followed by sections describing each in more detail;

1. Analyze numerous IHNV isolates in hand by RNase protection assays (RPAs) to generate fingerprints of IHNV throughout the Columbia River Basin.
2. Obtain more isolates as necessary and analyze them to create a thorough survey of the genetic types of IHNV throughout the Columbia River basin.
3. Begin archiving and comparing all data using a computerized database to identify links that provide evidence of virus traffic patterns and sources of disease outbreaks.

Objective 1. Analyze numerous IHNV isolates in hand by RNase protection assays (RPAs) to generate fingerprints of IHNV throughout the Columbia River basin.

In our two localized studies we have completed analysis of 37 IHNV isolates from the Deschutes River system and 81 isolates from Clear Springs facilities on the upper Snake River. Many fish health colleagues have already provided us with collections of IHNV isolates from various other locations in the Columbia River basin but very few have been analyzed due to the lack of personnel and funding to process them. These isolates cover a substantial portion of the Columbia River Basin;

Systemwide Collection of IHNV Isolates In Hand

Sub-region	Sub-basin	# Iso-lates	Year range	Host species[a]	Locations
Lower Col.	Lewis	2	'73, '80	ChF, ChS	Lewis River H.
	Willamette	18	'89-'97	ChS, Rb, StS, StW	Clackamas H., Minto Pond, Nan Scott Lk., South Santiam H., Foster Trap, Leaburg H.
Lower Mid-Columbia	Deschutes*	50*	'78-'98	ChS, StS, K	Wizard Falls, Metolius R., Link Ck., Opal Springs, Lk. Billy Chinook, Round Butte H.
	Hood	3	'95-'98	ChS, StW	Hood R., Parkdale H.
	Lt. W. Sal.	2	'87-'96	ChS, ChF	Little White Salmon H.
	Mainstem	8	'88-'97	ChS, ChF StS	Bonneville H., Carson NFH
	Umatilla	1	'95	StS	Minthorn
Upper Mid-Columbia	Entiat	3	'84-'89	ChS	Entiat H.
	Methow	3	'88-'97	ChS	Winthrop NFH
	Okanogan	3	'97-'98	Sock, K	Okanogan R., Okanogan Lk.
	Wenatchee	10	'82-'97	ChS	Leavenworth NFH
Lower Snake	Clearwater	2	'84, '88	StS	Dworshak NFH
	Grande Ronde	15	'86-'97	ChS, StS	Lookingglass H., Wallowa H, Big Canyon, Cottonwood
	Imnaha	3	'88-'91	ChS, StS	Little Sheep, Imnaha
	Salmon	2	'82	ChS, StS	Rapid River H., Pahsimeroi
Upper Snake	Up. Snake*	81*	'82-'98	Rb	Clear Springs Inc, 4 facilities
	Up. Snake	6	'78-'90	Rb	Rangen, Rimview

* indicates the samples already analyzed in the two initial studies described in section a. These are included for a complete picture of our coverage of the basin, but they are not included in the total number of isolates to be analyzed for objective 1.
[a] ChS and ChF (spring and fall chinook) are *Oncorhynchus tshawytscha*; StS, StW, and Rb (summer and winter steelhead and rainbow trout) are *O. mykiss*; Sock and K(sockeye and kokanee) are *O. nerka*.

These IHNV isolates were provided through collaborations with various colleagues in the U.S.G.S Biological Resources Division, U.S. Fish and Wildlife Service, Oregon Department of Fish and Wildlife, Canadian Department of Fisheries and Oceans, Clear Springs Inc., and Rangen Inc., as detailed in section c. Excluding the isolates already analyzed in the Deschutes and Clear Springs studies, we have 81 IHNV isolates in hand representing 27 different locations in 15 different sub-basins of the Columbia River basin. The majority of these (50 out of 81) were isolated recently, since 1990, and the rest provide a historical and temporal perspective of IHNV in the basin over the last 25 years. The collection contains IHNV isolated from three major host species, including both wild and hatchery populations. It also includes isolates from spawning adults as well as from disease epizootics in fry, smolts, and yearlings. The isolates from the Grande Ronde subbasin include virus from disease outbreaks in ESA listed Imnaha chinook stocks.

To accomplish objective 1, each of these isolates will be grown in tissue culture to prepare viral RNA, and each RNA will be assayed by RPA with each of the three probes described in section f. Each assay will be run at least twice to assure reproducibility. Where there are subtle differences in fingerprint patterns they may be run more times to facilitate all comparisons necessary for confidence in the analyses. Thus, this objective will entail running an estimated 500-800 assays.

Once the fingerprint patterns are generated they will first be assessed visually to determine the number of different genetic types identified by each probe, and then the groupings from each of the three probes will be used to determine the total number of composite genetic types present within the 81 isolates. Within these groupings we anticipate finding links between isolates from different locations and/or dates, that will indicate viral traffic patterns and potentially identify sources of strains responsible for disease outbreaks. Where multiple isolates are available from single locations we will assess changes in the virus types over time to determine the stability or the frequency of virus displacement by introductions from other sources. We will also assess indications of evolution in which a virus strain is displaced by a strain with an extremely similar but non-identical fingerprint pattern, such as could be generated by a single mutation.

Objective 2. Obtain more isolates as necessary and analyze them to create a thorough survey of the genetic types of IHNV throughout the Columbia River basin.

Although we have in hand a large collection of IHNV isolates representing many of the sub-basins affected by the virus, we will continue to establish collaborative interactions with fish health professionals and obtain additional isolates that would make our collection more complete. We would like to have better representation from the Lower Columbia, Upper Columbia, and Upper Snake sub-basins. For these purposes Dr. S. Gutenberger (USFWS, Underwood WA), Dr. K. Johnson (Idaho Dept. Fish and Game, Eagle, ID) and D. Ramsey (Rangen Inc.) have expressed willingness to send us virus isolates, and we will contact additional colleagues as analyses proceed. Any additional isolates will be analyzed as described above in objective 1.

Objective 3. Begin archiving and comparing all data using a computerized database to identify links that provide evidence of virus traffic patterns and sources of disease outbreaks.

Although we have identified several links between IHNV isolates from different sources by manual visual comparison, the volume of data that will be generated in this study will require computer assisted analysis to assure that links are not overlooked. For this reason we have purchased a computer program for archiving and comparing fingerprint patterns, the “Molecular Analyst Fingerprint Analysis” program from Biorad Inc. This program archives fingerprint patterns in an annotated, searchable database that can be directed to do comparisons to identify any identical patterns, or patterns with designated levels of similarity.

The fingerprint patterns representing each genetic type identified in objectives 1 and 2 will be entered into this computer program, along with the patterns from our Deschutes and Clear Springs studies, to create a large database of all the genetic types of IHNV throughout the Columbia River Basin. This database will provide a baseline of information that can be expanded in the future as any new IHNV

isolates are analyzed. Once archived in this form, we will direct the program to search the entire database for any identical fingerprint patterns, or any patterns with a high level (a specified % of identical bands in the patterns) of similarity. All links thus identified will be used to infer virus traffic patterns and sources of disease outbreaks as described for manual comparisons in objective 1. This information will be provided to fish health professionals and resource managers in hopes that a better understanding of the sources of IHNV within their regions or facilities will assist them in designing strategies to reduce the impact of IHNV and improve the overall health of their salmon populations. This information may also be used to assist management decisions to reduce risks involved in stocking and movement of fish stocks, along with their associated virus strains, within the basin.

The overall traffic patterns elucidated will be interpreted to provide insights into the mechanisms and causes of virus movements and changes throughout the basin. Possible factors include phenomena such as the known stray rates of salmonids, the biological life cycles of the salmonid hosts, climatic factors such as flooding or temperature variations, and anthropogenic causes such as environmental modifications or fish culture activities.

In practical terms the final product of this objective will be a database that provides a thorough baseline of information about the genetic types of IHNV throughout the Columbia River basin. Once established, this database can be used in the future to provide relevant comparisons for any newly isolated IHNV associated with unusual biological attributes or novel disease outbreaks. Due to the one year nature of this proposal we plan to initiate the database described above, and carry out analyses as far as possible, but it is likely that completion of this objective will extend beyond the time designated. In that event it will be completed using alternative funding.

f. Methods

The IHN virus isolates described in this proposal will be analyzed by RNase protection analyses (RPA) to generate diagnostic fingerprint patterns that indicate genetic differences and relatedness between virus strains. Although most studies of genetic diversity and virus traffic in mammalian viruses have been done using nucleotide sequence data, we have found that this method is not optimal for field isolates of IHNV. In our studies using 300-700 nucleotide lengths of sequence data we have found that the levels of divergence between IHNV field isolates within a watershed are often not sufficient to generate phylogenetic relationships with significant confidence values (Kurath, Anderson, and Emmenegger, in prep). Since this severely limits any conclusions from sequence data, we have selected RPA as a more informative method to establish traffic patterns for IHNV.

The RPA methods developed in our laboratory are based on published protocols (Winter et. al., 1985) that have been adapted to analysis of IHNV (Kurath et. al., 1995). We use plasmid clones containing three of the virus genes, the G, N, and NV genes, to produce molecular probes that detect sequence differences in these genes. These probes are synthesized by making minus-sense RNA copies of these genes containing radioactive uracil. Together these probes assess over 3000 nucleotides (30%) of the virus RNA genome for genetic variation. Details of probe synthesis and the RPA assay protocol are available (Kurath et. al., 1992, 1995) and the method is described briefly below.

IHNV isolates to be assayed are used to infect tissue culture cells of the *epithelioma papulosum cyprini* (EPC) cell line and viral RNA is extracted from infected cells using a guanidinium HCl procedure. This plus-sense viral RNA is hybridized with each minus-sense RNA probe to produce complemetary double-stranded duplexes. These duplexes are digested with RNase enzymes under conditions where many sequence mismatches will be cleaved, generating a set of cleavage products from the probe. These cleavage products are visualized by separation on polyacrylamide gels, and each product produces a band detected by placing X-ray film on the gel. Thus, with each probe, each virus isolate produces a set of bands of different lengths that comprises a fingerprint pattern. Since these cleavage product bands result from genetic sequence differences, isolates with identical fingerprint patterns are genetically extremely similar, and isolates with similar but non-identical patterns are related. The number of shared and unique bands between any two fingerprint patterns is an indication of the genetic relatedness of the isolates they represent.

This RPA methodology is in routine use in this laboratory, so no difficulties are anticipated with producing fingerprint patterns from the IHNV isolates described. Due to the use of cell culture this proposal involves no animal studies, and no risks to habitats or humans. The only potential source of

difficulty may be in implementing the computerized Fingerprint Analysis program, since we have not yet used it extensively. However, the capabilities described for this program are ample to accomplish the objectives we have described, and we have archived fingerprint data from other studies successfully. Therefore, we are confident that any difficulties that arise in using the program as described can be overcome.

g. Facilities and equipment

The Western Fisheries Research Center (WFRC) is a federal research laboratory of the USGS Biological Resources Division, located at 6505 NE 65th St. in Seattle. The laboratory has been active in fish microbiology research since its establishment in 1934 as part of the national Fish Hatchery Disease Service,and it has a long-standing record of service to the U.S. Fish and Wildlife Service. The dry laboratory and wet laboratory buildings completed in 1994 represent state-of-the-art facilities for fish microbiology research. The dry laboratory has more than 16,000 square feet of laboratory space with facilities for virology, bacteriology, cell culture, molecular biology, immunology, and histology. The laboratories are well equipped with ultracentrifuges, refrigerated centrifuges, ultra-freezers, DNA synthesizer, electrophoresis equipment, spectrophotometer, scintillation counter, laminar flow hoods, and all capital equipment needed to perform the work described in this proposal. In the dry laboratory building there is a radiation laboratory for working with radioisotopes. This facility is fully licensed and in compliance with all regulations of the federal Nuclear Regulatory Commission (NRC). In addition, the PI for this proposal is the official radiation safety officer for WFRC, and is responsible for assuring that all NRC requirements are met. With regard to this specific proposal, it is significant that the Molecular Analyst Fingerprint Analysis software and scanner ($12,000 total) have already been purchased and that the annual radiation license fee of $5,500 is paid by base funds. Although the center has adequate computer capability for all its current functions, the large database and complex analyses to be performed as part of objective 3 make it desirable to acquire one new computer that will be dedicated to this project and have ample hard drive and RAM space for future needs.

h. Budget

The principle investigator for this proposal, Dr. G. Kurath, is an employee of the USGS Biological Resources Division and this proposal will require approximately 30% of her effort (0.3 FTE). In addition, E.J. Emmenegger, a permanent staff research microbiologist in this program, will also contribute approximately 30% of her effort (0.3 FTE) to this project. No salary support is requested for these contributions. The main request is for funds to hire a full-time GS9 level technician with experience in virology and molecular biology. The starting salary for a GS-09 research technician in the Seattle area is set by the federal government at $32,478 per year. The standard benefits rate of 25% for a term temporary position is also set by the federal government. We are requesting $10,000 for one year of supplies, which will include radioactive isotope (approximately one order per month, at $200 per order), cell culture media and supplies, assay reagents and enzymes, X-ray film, electrophoresis supplies, one set of pipettors, and disposables such as pipette tips, tubes, and gloves. A single item of equipment is requested, which is a MacIntosh G3 computer that will be dedicated to the Fingerprint Analysis program database described in objective 3. This specific computer is requested to interface with our existing data sets and computer capability, and because the size of this database will be extremely large due to the complex fingerprint patterns that will be archived and analyzed as scanned images. A small amount, $400, is requested under operations to pay fedex charges for our collaborators who routinely send us virus isolates packaged on dry ice. Due to the acquisition of virus isolates by collaboration with colleagues we do not include funds for field sampling. However, occasionally a colleague will request that one of us accompany them on a trip to the field to assist with collection of multiple samples that are not part of their routine duties. Although we do not have any such requests at present, we have included $1000 under travel to pay for one or two trips that we anticipate may arise in connection with objective 2. There is also $500 requested under travel to pay for one trip to present results of this project at the Western Fish Disease Conference in the year 2000. Finally, the WFRC indirect cost rate is 38%.

Section 9. Key personnel

Dr. G. Kurath, the principle investigator for this proposal, is a GS13 Research Microbiologist with the USGS-Biological Resources Division. I anticipate that I will devote approximately 30% (0.3 FTE) of my time to this project. I will be responsible for hiring and training the technical personnel requested, and I will supervise this technician in all objectives of the work. I will also be directly involved with generating the data, analyzing and interpreting the results, and with implementing the computerized database for archiving and analyzing the fingerprints. I have ten years of experience working with IHN virus, and I have twelve years of experience with applying RNase protection technology to studies of the genetic diversity of various RNA viruses, including viruses of plants. My resume is provided below.

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EDUCATION

B. A. - 1978, Miami University, Oxford, Ohio (Microbiology, cum laude)
M.S. - 1980, Oregon State University (Marine microbiology)
Ph.D. - 1985, Oregon State University (Microbiology, virology)

EMPLOYMENT

Postdoctoral Research Associate -1985-1988
Cornell University, New York
Postdoctoral Research Associate - 1988-1989
Waite Agricultural Research Institute, Adelaide, Australia
Postdoctoral Research Associate - 1989-1992
University of California, Riverside, California
Research Microbiologist , GS12- 1992-1996
NBS Northwest Biological Science Center, Seattle, Washington
Assistant Professor of Pathobiology, affiliate- 1994 to present
University of Washington, Seattle, Washington
Research Microbiologist , GS13- 1997-present
BRD USGS Western Fisheries Research Center, Seattle, Washington

CURRENT RESPONSIBILITIES

Develope and sustain an active research program on significant pathogens of fish in Western North America
Supervision of one technician, one post-doctoral researcher, and one graduate student
Radiation safety officer for the Western Fisheries Research Center

EXPERTISE

Molecular biology and microbiology of RNA viruses, with an emphasis on virus epidemiology, genetic diversity, evolution, and control.

FIVE RELEVANT PUBLICATIONS:

1. **Kurath, G.**, and Leong, J. C. (1985). Characterization of IHN virus mRNA species reveals a non-virion rhabdovirus protein. J. Virol. 53:462-468.

2. **Kurath, G.**, Heick, J., and Dodds, J. A. (1993) RNase protection analyses show high genetic diversity among field isolates of satellite tobacco mosaic virus. Virology 194:414-418.
3. **Kurath, G.**, Higman, K.H., and Bjorklund, H.V. (1995). The NV genes of fish rhabdoviruses:development of RNase protection assays for rapid assessment of genetic variation. Vet. Res. 26:477-485.
4. Bjorklund, H. V., Higman, K. H., and **Kurath, G.** (1996). The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus : analysis of relationships with other rhabdoviruses. Virus Res. 42:65-80.
5. **Kurath, G.**, Higman, K.H., and Bjorklund, H. V. (1997). Distribution and variation of NV genes in fish rhabdoviruses. J. Gen. Virol. 78:113-117.

Section 10. Information/technology transfer

The information on IHN virus genetic types, traffic patterns, and sources of disease outbreaks obtained from this project will be communicated directly with colleagues who have supplied the virus isolates, and it will be presented to the Columbia Basin fish health research community at the Western Fish Disease workshop in 2000. We anticipate several publications in peer-reviewed journals from this work once the entire database is complete. We will continue to host RPA training visits, and the database created will be accessible to any interested colleagues. Eventually we plan to make the entire database available as metadata on a server that can be accessed by any interested parties from remote locations. While this is beyond the scope of this first year proposal, we are committed to accomplishing this goal in the future.

In the meantime, once the baseline database is created, we will provide for its utility to any interested fish health professionals by being available, through collaborations, to analyze critical IHNV isolates that they provide. We will then compare the new fingerprints with the database to learn how they relate to the known background of genetic types in the Columbia River basin, and provide the colleagues with the most useful information possible.

Congratulations!